

Tougu Xiaotong capsule exerts a therapeutic effect by improving knee meniscus in the early osteoarthritis rat model

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Received October 25, 2019; Accepted November 25, 2019

DOI: 10.3892/etm.2020.8624

Abstract. The aim of the study was to observe the effects of Tougu Xiaotong capsule (TGXTC) on the microstructure and ultrastructure of meniscus in rats with early knee osteoarthritis (KOA). A total of 27 Sprague Dawley rats were randomly divided into three groups: The normal group (non-papain-induced KOA; received saline only), the model group (papain-induced KOA; received saline only) and the TGXTC group [papain-induced KOA; received TGXTC (0.31g·kg⁻¹·d⁻¹)]. After 4 weeks treatment, the animals were anesthetized and the sagittal plane of the intact knees (n=6 per group) was obtained and prepared in paraffin section. Following hematoxylin and eosin staining, the degeneration of cartilage structure was evaluated via Mankin score, the microstructure of meniscus was observed and the area of calcification in meniscus was analyzed. Following toluidine blue staining, the content of proteoglycan in meniscus was analyzed. Three samples in each group were obtained and the ultrathin sections of meniscus were observed through a transmission electron microscope. The results showed that compared with the normal group, in the model group the joint space became narrow and the cartilage layer was slightly damaged and the Mankin score was 4.17±0.76, suggesting that the early KOA model was successfully established. After TGXTC treatment, the joint space stenosis and cartilage damage were improved

as the Mankin score significantly decreased. Compared with the normal group, in the model group the surface of meniscal cartilage was much more uneven, the area of calcification was significantly increased and the content of proteoglycan of cartilage matrix was significantly decreased. However, following TGXTC treatment, the surface of the meniscal cartilage was much more smooth and flat, and the damage of tissue structure and the calcified area were significantly reduced, and the proteoglycan of cartilage matrix content was significantly increased. Compared with the normal group, the number of cellular processes and organelles, including the rough endoplasmic reticulum, mitochondria and Golgi apparatus of meniscal cartilage were reduced and swollen in the model group. In addition, the nuclei were deformed and heterochromatin agglutinated. The extracellular collagen fibrils became slender, disordered and sparse. Compared with the model group, the TGXTC group had more cell processes and organelles, alleviated swelling and heterochromatin agglutinating. Additionally, the collagen fibrils around the cells were thicker, larger and arranged in an orderly manner. In conclusion, TGXTC exerted its therapeutic effects on the development of KOA via reducing the destruction of the cartilage structure of the meniscus and improving the composition and function of the meniscus cartilage matrix.

Introduction

The menisci are crescent-shaped wedges of fibrocartilage located on the medial and lateral aspects of the knee. The primary function of the meniscus is to transmit load across the tibiofemoral joint by increasing congruency, thereby decreasing the resultant stress placed on the articular cartilage, and to play a role in shock absorption, stability, lubrication, nutrition, and proprioception to the knee joint (1-8). As a consequence of its complex anatomical, biomechanical, and functional characteristics, the menisci are prone to damage and injury (9). Injuries to the menisci are recognized as a cause of significant musculoskeletal morbidity (10,11). Previous findings have shown that knee injury is one of the strongest risk factors for the

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Key words: meniscus, knee osteoarthritis, Tougu Xiaotong Capsule, microstructure, ultrastructural, proteoglycan

development of knee osteoarthritis (KOA) (12,13). Therefore, the challenge is to develop therapies and techniques with the aim of preserving the meniscus's distinct composition and function, thereby delaying the development of knee osteoarthritis.

Tougu Xiaotong capsule (TGXTC) is an effective prescription in the treatment of KOA. TGXTC comprises *Ligusticum chuanxiong*, *Morinda officinalis*, *Sarcandra glabra* and *Paeonia lactiflora* (14,15). Numerous efficacious molecules are present in TGXTC, which constitutes multi-drug, multi-path and multi-target molecular mechanisms for the treatment of KOA, as well as the non-linear regulation pattern existing in TGXTC ligand-target interaction network (16). Previous findings have demonstrated that TGXTC has therapeutic effects on KOA (17) via multiple targets, including the inhibition of chondrocyte apoptosis (18), amelioration of the structure and function of cartilage (19), arresting cartilage degradation (15), promoting osteoblast proliferation and calcium secretion (14), and regulation of subchondral bone remodeling (20). However, the effect of TGXTC on meniscus has not been reported.

In the present study, the effect of TGXTC on the microstructure and ultrastructure of meniscus in KOA was observed, so as to provide the experimental proofs for the application of TGXTC in the clinical treatment of meniscus injury in KOA.

Materials and methods

Animals. A total of 27 male, 6-week-old SPF Sprague-Dawley (SD) rats, qualified number SCXK (Hu) 2017-0005, were purchased from the Shanghai Slack Laboratory Animal Co. (Shanghai, China). The rats were raised in the Animal Experimental Center of Fujian University of Traditional Chinese Medicine (permit no. SYXK 2014-0006; Fujian, China) at a room temperature of 20-26°C, a relative humidity of 40-70%, a 12 h light/dark cycle and free access to food and water. The care and use of the laboratory animals complied with the Guidance Suggestions for the Care and Use of Laboratory Animals 2006 of the Ministry of Science and Technology, China.

Drugs and reagents. TGXTC was prepared by the Second People's Hospital of Fujian University of Traditional Chinese Medicine (Fujian, China) (approval no. MIN ZIZHI Z20100006).

Experimental design. After one week of acclimation, 27 rats were randomly divided into three groups: The normal group (non papain-induced KOA; received equivalent amount of saline only), the model group (papain-induced KOA by an injection of 0.2 ml 4% papain solution on days 1, 4 and 7; received an equivalent amount of saline only) and the TGXTC group [papain-induced KOA by an injection of 0.2 ml 4% papain solution on days 1, 4 and 7; received a clinical oral dose of TGXTC (0.31 g/kg/day)]. All the groups were treated once daily for four consecutive weeks, after which the animals were anesthetized by intraperitoneal injection 10% chloral hydrate, then the sagittal plane of the intact knee (6 rats in each group) and meniscus (3 rats in each group) were obtained and prepared into paraffin section. Following hematoxylin and eosin (H&E) staining, the structure changes in cartilage

and meniscus were observed and the area of calcification was analyzed. The content of proteoglycan in meniscus was analyzed according to toluidine blue staining. The ultrathin sections of the meniscuses were observed through a transmission electron microscope (TEM).

Histology. The intact knee tissues were fixed in 4% paraformaldehyde for 3 days and decalcified in 10% EDTA at room temperature for approximately 8 weeks. The intact knee was longitudinally cut and embedded in paraffin. Sagittal sections (4 μ m) were prepared for H&E staining and toluidine blue staining and observed under an optical microscope (DM4000B; Leica Microsystems GmbH) and images were captured.

Mankin score. Following H&E staining, a modified Mankin scoring principles was used to evaluate the degeneration of the cartilage structure (21). According to the Mankin scoring principles, the total score was 14 points; 1-5 point was identified as early osteoarthritis, 6-9 point was identified as middle osteoarthritis, and 10-14 point was identified as late osteoarthritis.

Microscopic image analysis. According to the H&E staining and toluidine blue staining results, the area of calcification and the content of proteoglycan in meniscus were assessed by image analysis system (Motic Med 6.0) respectively.

Transmission electron microscopy analysis. The meniscus specimens were cut into 2.5x1x1 mm blocks, pre-fixed in 3% glutaraldehyde [Alfa Aesar (China) Chemicals Co., Ltd.] and 1.5% paraformaldehyde solution (pH 7.3) at 4°C for 4 days, post-fixed with 1% osmium tetroxide (Ted Pella, Inc.) at 4°C for 2 h following decalcification in 10% EDTA for one week at 4°C. The tissue specimens were dehydrated with graded alcohol-acetone and embedded in Epoxy resin 618 (E-51, Ganxi Chemical Co. Ltd.). The 1- μ m resin semi-thin sections were subsequently cut using a microtome and stained with azur-methylene blue. The structure of the meniscus was observed under the optical microscope (DM4000B; Leica Microsystems GmbH). The 70-nm ultrathin sections were cut using an ultramicrotome (EM UC6; Leica Microsystems GmbH), stained with 2% aqueous uranyl acetate and 0.3% lead citrate. The ultrastructure of the meniscus was observed using a transmission electron microscope (H7650; Hitachi, Ltd.) at 80 kV.

Statistical analysis. Experimental data were processed and analyzed using SPSS 22.0 software (IBM Corp.). The Shapiro-Wilk test was used to determine the normality of all groups of data. If the data exhibited a normal distribution, they were analyzed with one-way analysis of variance followed by the least significant difference or Games Howell post hoc tests. If data did not exhibit normal distribution, the Kruskal-Wallis test was used and the Mann Whitney U with Bonferroni's correction was applied as the post hoc test. $P < 0.05$ was considered to indicate a statistically significant difference.

Results

Joint microstructure and Mankin score. As shown in Figs. 1 and 2, in the normal group, the joint space was uniform; the surface was smooth and the four-layer structures (including surface layer, transitional layer, radiation layer and calcifica-

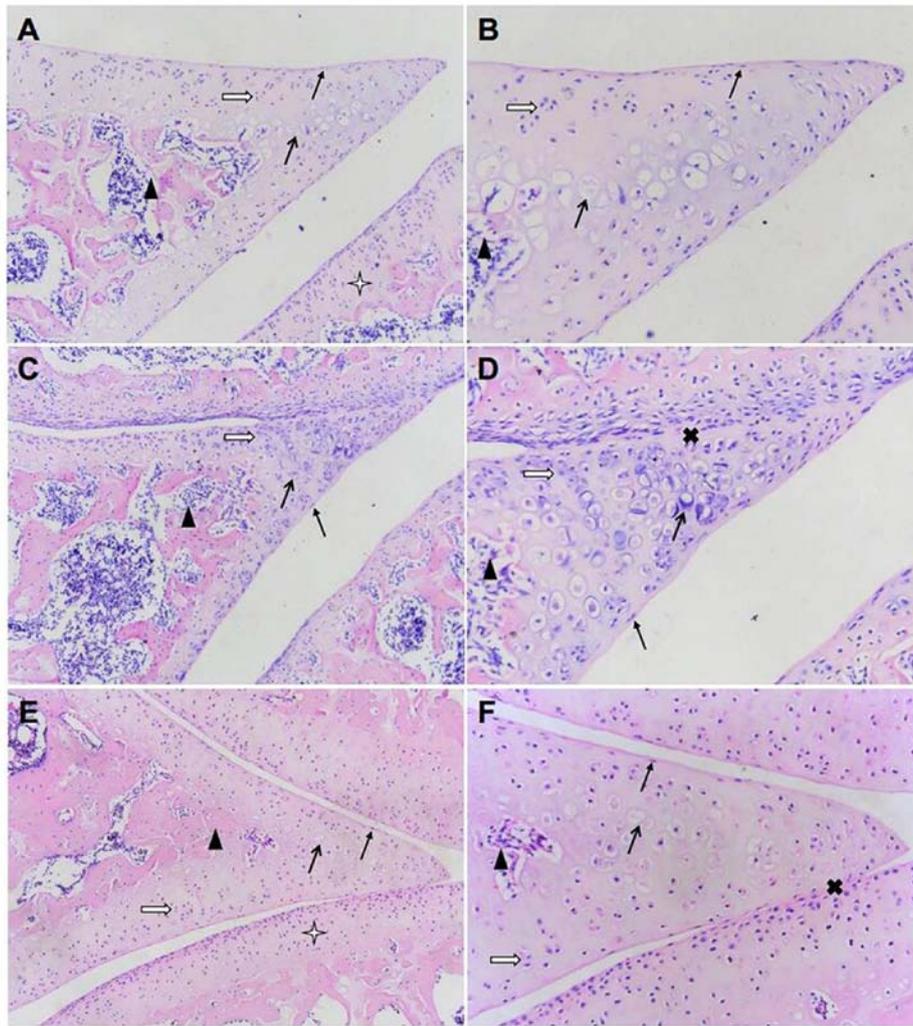


Figure 1. Effect of TGXTC treatment on the microstructure of cartilage tissue. Following treatment with or without TGXTC for four consecutive weeks, histopathological alterations were evaluated by hematoxylin and eosin. The morphological changes in the cartilage were observed under a microscope and images were acquired at a magnification of (A, C and E) x100 or (B, D and F) x200. (A) In the normal group, the joint space was uniform, (B) the surface was smooth and the articular cartilage structures were regular. (C) In the model group, the joint space was uneven with local stenosis and adhesion, (D) the surface of articular cartilage was uneven and the structure was disordered. (E) In the TGXTC group, the narrow joint space and adhesion areas were reduced, (F) the cartilage surface was smoother and the cartilage structure was relatively in order. →, surface cells; ⇨, isogenous group; ⇒, calcified cells; ▲, subchondrial bone; ✱, adhesion; ✧, articular cartilage.

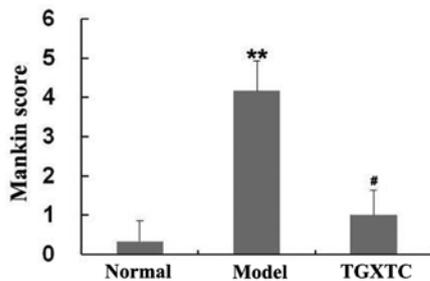


Figure 2. Effect of TGXTC treatment on the Mankin score in cartilage tissue. Following H&E staining, the histological grading was evaluated by a modified Mankin scoring principles. The Mankin score in the model group was significantly higher than that in the normal group. However, it was significantly lower in the TGXTC group than the model group. **P<0.01 vs. the normal group; #P<0.05 vs. the model group.

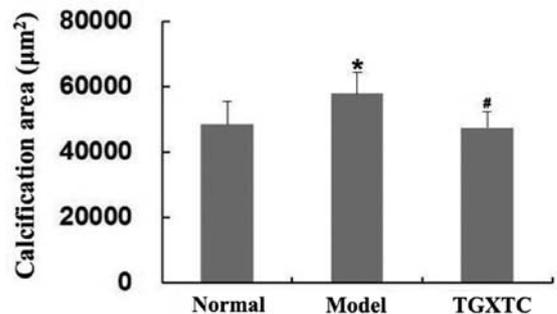


Figure 3. Effect of TGXTC treatment on the calcified area in meniscus. Following H&E staining, the calcified area in meniscus was evaluated. Calcification area in the model group was significantly higher than that in the normal group. However, it was significantly lower in the TGXTC group than the model group. *P<0.05 vs. the normal group; #P<0.05 vs. the model group.

tion layer) of articular cartilage were arranged regularly with the Mankin score: 0.33±0.52. Compared with the normal

group, the model group showed the joint space was uneven with local stenosis or even adhesion; the surface of articular

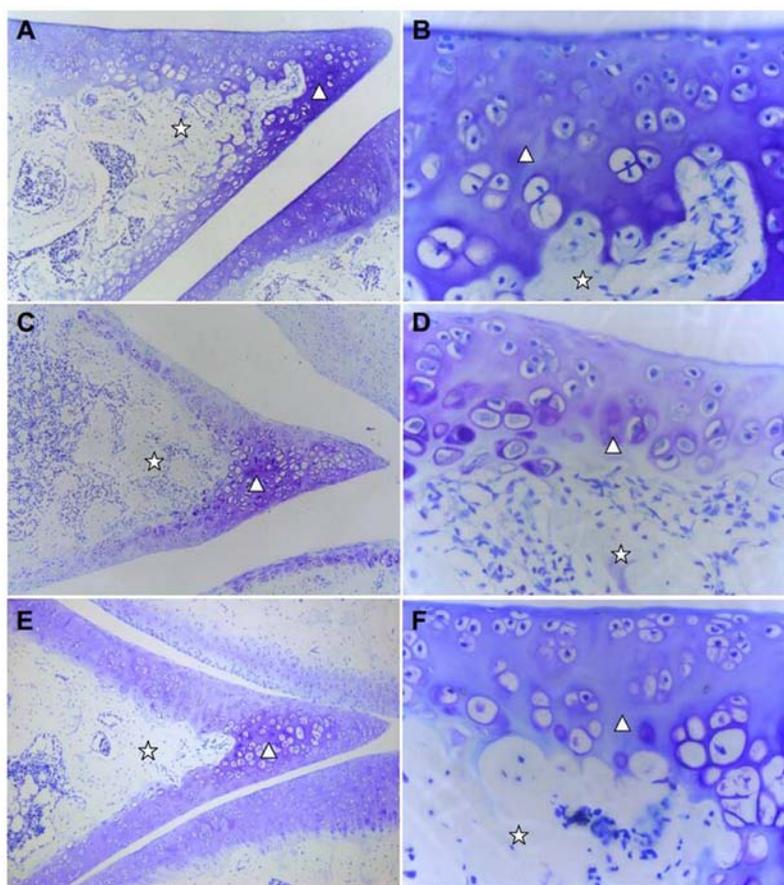


Figure 4. Effect of TGXTC treatment on the proteoglycan in meniscus cartilage matrix. Following treatment with or without TGXTC for 4 consecutive weeks, proteoglycan alterations were evaluated by toluidine blue staining. The changes in the meniscus cartilage matrix were observed under a microscope and images were acquired at a magnification of (A, C and E) $\times 100$ or (B, D and F) $\times 400$. (A) In the normal group, the meniscus cartilage matrix was stained dark purple-blue and (B) the color was uniform. (C) In the model group, the staining intensity of the cartilage matrix was markedly decreased, (D) with some areas unstained (E) In the TGXTC group, the meniscus cartilage matrix was deeply stained and (F) the color was relatively uniform. Δ , proteoglycan in meniscus cartilage matrix; \star , subchondral bone.

cartilage was uneven and the four layers were disordered; the number of chondrocytes in the surface layer was decreased or even lost in some areas; the number of chondrocytes in the transitional layer and the radiation layer appeared to be scarce, but the cells in the calcified layer were relatively increased (Fig. 1).

The Mankin score in the model group was 4.17 ± 0.76 , which was significantly increased compared to the normal group (0.33 ± 0.52 , $P < 0.05$), suggesting that the early osteoarthritis model was successfully established, which was consistent with our previous results (22). After treatment with TGXTC, the narrow joint space and adhesion areas became relatively fewer, the cartilage surface smoother and the thicker cartilage layer was clearly visible compared to those in the model group. The chondrocytes status was larger and oval or round in both the transitional layer and in the radiation layer or in the calcification layer, and the nucleus in these layers were more and clearly visible as evidenced by the significantly higher Mankin score with 1.00 ± 0.63 ($P < 0.05$) (Fig. 2).

Microstructure observation of meniscus. As shown in Fig. 1, the surface of cartilage in meniscus was smooth, and the chondrocytes were regularly distributed. The surface chondrocytes were flat with diffused distribution. The middle layer cells

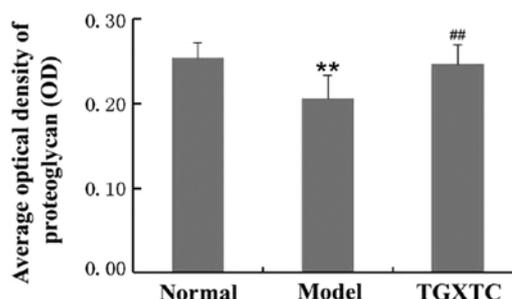


Figure 5. Effect of TGXTC treatment on the average optical density of proteoglycan in meniscus cartilage matrix. After toluidine blue staining, the average optical density of proteoglycan was evaluated. In the model group, it was significantly lower than that in the normal group., but significantly higher in the TGXTC group than the model group. ** $P < 0.01$ vs. the normal group; ** $P < 0.01$ vs. the model group.

were larger, forming 2-4 isogenous group. The deep layer cells were hypertrophic, which were near the subchondral bone. Cartilage lacunae were deeply stained, and part of the cell nuclei were not clear or disappeared, showing as calcified cells, i.e., temporary calcified areas. However, in the model group, the surface of meniscus was uneven and locally adhered

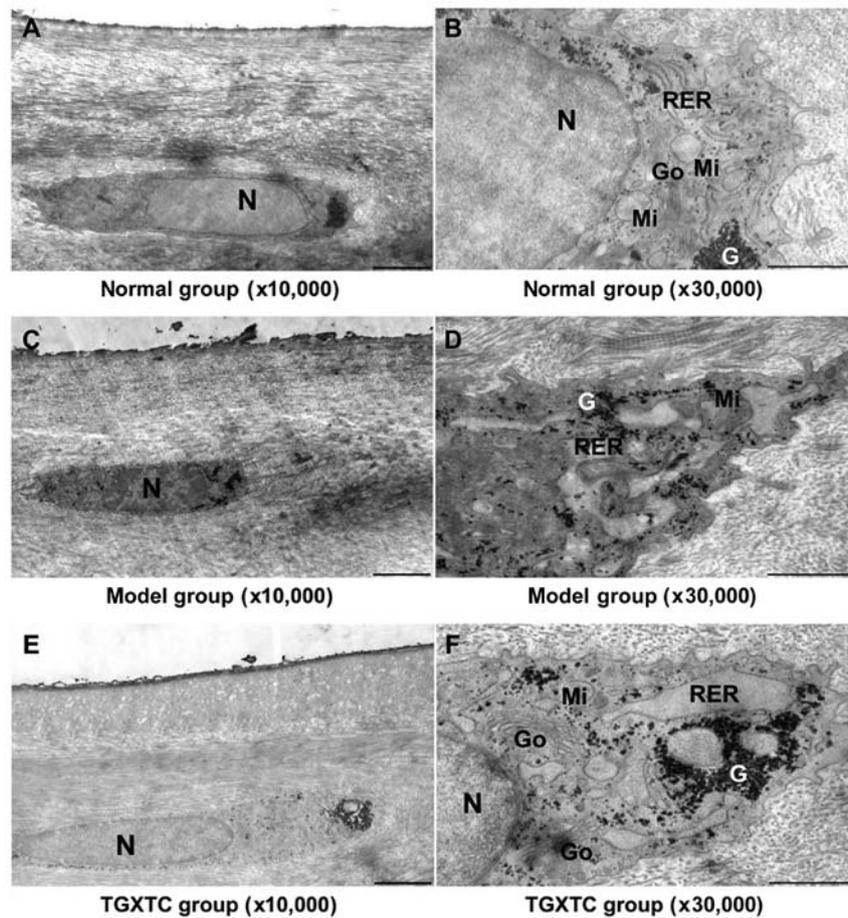


Figure 6. Effect of TGXTC treatment on the ultrastructure of the cell in the superficial layer of meniscus. Following treatment with or without TGXTC for 4 consecutive weeks, the ultrastructure of the cells in the superficial layer of meniscus was observed under a transmission electronic microscope and images were acquired at a magnification of (A, C and E) $\times 10,000$ (scale bar, $2\ \mu\text{m}$) or (B, D and F) $\times 30,000$ (scale bar, $1\ \mu\text{m}$). (A) In the normal group, the cells were fusiform with few and short processes and little cytoplasm, and (B) the nuclei were elliptic with uniform chromatin. (C) In the model group, the nuclei were deformed and the heterochromatin was agglutinated, and (D) the number of organelles was reduced and they were swollen. (E) In the TGXTC group, the nuclear morphology was similar to in the normal group and (F) organelle swelling was alleviated. TGXTC, Tougu Xiaotong capsule; N, nuclei; RER, rough endoplasmic reticula; Go, Golgi apparatus; Mi, mitochondria; G, glycogen.

to articular cartilage. The boundary between articular cartilage and meniscus was blurred. The cartilage layer became thinner. The surface cells were very scarce, the middle layer chondrocyte proliferation was obvious, homologous cells were aggregated, the number of hypertrophic chondrocyte in the deep layer was increased, and the nuclei of some cells in the cartilage lacunae were not clear or absent.

Quantitative analysis of calcified area in meniscus. As shown in Fig. 3, compared with the normal group (the calcified area was $48,406 \pm 6,943\ \mu\text{m}^2$), the surface of meniscal cartilage in the model group was uneven and the calcified area was significantly increased to $57,795 \pm 6,521\ \mu\text{m}^2$ ($P < 0.05$). Compared with the model group, the surface of the meniscal cartilage in the TGXTC group was smooth and flat, and the damage of tissue structure was reduced, leading to the calcified areas being significantly decreased at $47,423 \pm 5,051\ \mu\text{m}^2$ ($P < 0.05$). In the TGXTC group, the surface cells were scattered and the thickness of the perichondrium was the same. The middle layer cells formed homologous cell groups with 2-3 cells. The deep layer cell proliferation was lower than that of the model group.

Quantitative analysis of proteoglycan in the meniscus cartilage matrix. Toluidine blue staining results showed that the meniscus cartilage matrix in the normal group was uniformly dyed with dark purple-blue, while the color depth in the model group was significantly decreased, showing light blue dye, whereas some areas were unstained (Fig. 4). Image quantitative analysis revealed that the optical density in the model group (0.2060 ± 0.0270) was significantly decreased compared to that in the normal group at 0.2537 ± 0.0185 ($P < 0.01$). Compared with the model group, the meniscus cartilage matrix in the TGXTC group was deeply stained and uniform, and the optical density was significantly increased to 0.2463 ± 0.0230 ($P < 0.01$) (Fig. 5).

Ultrastructural observation of meniscus. In the normal group, most of the cells in the superficial layer of meniscus were fusiform with few and short processes and little cytoplasm, and most of the cells in the inner layer were triangular-like with more processes and abundant cytoplasm, rich in the rough endoplasmic reticulum, mitochondria and Golgi apparatus. The extracellular collagen fibrils were mainly type II collagen fibrils of $35 \pm 5\ \text{nm}$, and were ordered and densely

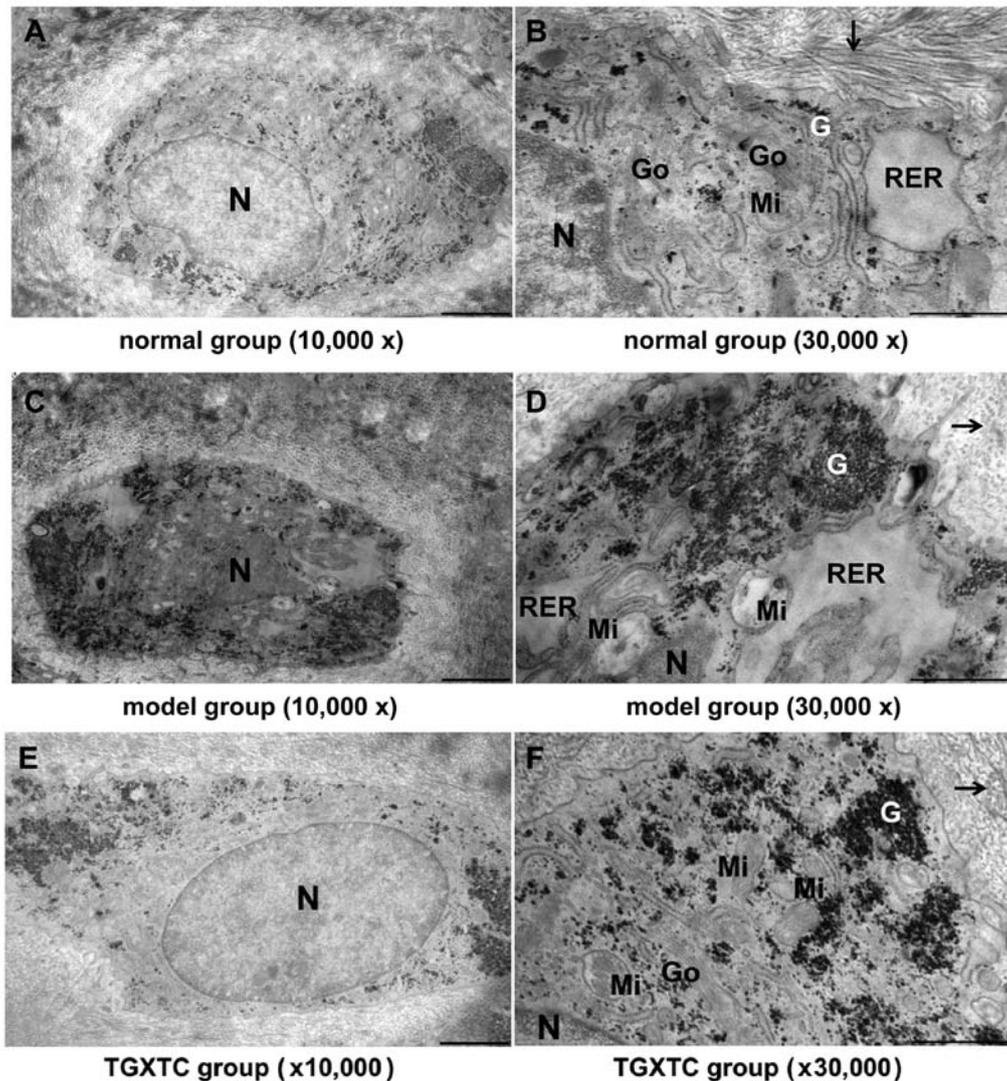


Figure 7. Effect of TGXTC treatment on the ultrastructure of the cells in the inner layer of meniscus. Following treatment with or without TGXTC for 4 consecutive weeks, the ultrastructure of the cells in the inner layer of meniscus was observed under a transmission electronic microscope and images were acquired at a magnification of (A, C and E) $\times 10,000$ (scale bar, $2 \mu\text{m}$) or (B, D and F) $\times 30,000$ (scale bar, $1 \mu\text{m}$). (A) In the normal group, the cells had abundant processes and cytoplasmic organelles, and (B) the extracellular matrix contained ordered and densely packed type II collagen fibrils. (C) In the model group, the number of processes was reduced, the nuclei were deformed and the heterochromatin was agglutinated, (D) the number of cytoplasmic organelles was reduced and they were swollen with accumulated glycogen, and the extracellular collagen fibrils become slender, disordered and sparse. (E) In the TGXTC group, the cell processes were increased and the nuclear morphology was similar to in the normal, (F) organelle swelling and glycogen accumulation were alleviated, and the thickness and size of collagen fibrils were increased and orderly arranged. Black arrows indicate type II collagen fibril; TGXTC, Tougu Xiaotong capsule; N, nuclei; RER, rough endoplasmic reticula; Go, Golgi apparatus; Mi, mitochondria; G, glycogen.

packed. Compared with the normal group, in the model group, the numbers of processes in the superficial and inner layers of meniscal cartilage and organelles were reduced. The cells were swollen, the number of cytoplasmic organelles was reduced and swollen, glycogen was accumulated, and most of the nuclei were deformed and heterochromatin agglutinated. Extracellular collagen fibrils become slender ($25 \pm 5 \text{ nm}$), disordered and sparse. Compared with the model group, the TGXTC group had an increased number of cell processes, rough endoplasmic reticulum, mitochondria and other organelle swelling were alleviated, glycogen accumulation was alleviated, nucleus morphology was approximate to normal, the thickness and size of collagen fibrils around the cells were increased, and arranged in a relatively orderly manner (Figs. 6 and 7).

Discussion

Prevention and treatment of meniscus injury is significant to prevent the development of osteoarthritis. In early research, there was a focus on the role of articular cartilage in the pathogenesis of osteoarthritis. With advances in research, increasing attention has been given to the role of subchondral bone and synovium (23,24). However, research regarding the role of meniscus in osteoarthritis is relatively rare. Mechanical loading is critical to joint health. However, abnormal mechanical loading can lead to the onset and progression of osteoarthritis (OA) (25). Specifically, OA results from an imbalance of anabolism and catabolism in the joint, which may be influenced by the biological and mechanical environment (26). Thus, biomechanical studies

are critical to understanding OA development, prevention, and treatment. The menisci are integral to the normal function of the knee joint and play an important role in load distribution, shock absorption, stability, lubrication, and proprioception (1). If meniscus is injured or the location was changed, it can easily lead to knee instability and biomechanical changes, and damaged cartilage and other structures, ultimately lead to osteoarthritis (9). Therefore, the prevention and treatment of meniscus injury is very important in delaying the occurrence and development of osteoarthritis.

Current situation of meniscus injury treatment and the unique role of traditional Chinese medicine. Previously, treatment of meniscus injury was mainly based on excision. However, with the improvement in understanding the anatomical structure and physiological function of the meniscus, its importance was recognized and meniscus retention became imperative (27). In addition, owing to the particular supply of meniscus blood, the marginal part of the meniscus with rich blood supply healed well after suture, while repair of the central free edge, which was distant from the blood supply, was unsatisfactory (28,29). Therefore, conservative treatment was studied to promote meniscus healing. Nowadays, the conservative treatment of meniscus injury occurs mainly through intra-articular injection of growth factor or active ingredients in the extracellular matrix (30). In recent years, tissue-engineering research has led to the promotion of meniscus repair through construction of scaffolds and culturing stem cells, but the majority of such studies were in the pre-clinical experimental stage (31,32). Previous studies had proven that Traditional Chinese Medicine could play a unique role in conservative treatment. For example, Cheng *et al* (33) found that the addition or subtraction of Taohong Siwu Decoction and Wuling Powder could promote the rehabilitation of joint function after meniscus injury surgery. Tang (34) found that compound Longxuejiesan powder had an ideal therapeutic effect on an acute meniscus injury. Song and Li (35) found that Huoxue Xiaozhong Decoction treatment for meniscus injury patients receiving arthroscopic meniscus plasty and suture could relieve pain and promote the recovery of joint function.

Repair effect of TGXTC on meniscus tissue structure. Although significant progress has been made in understanding KOA pathophysiological pathways, much remains to be done to develop a specific therapy that could effectively retard or prevent the progression of the disease. To this end, the literature suggests that in addition to cartilage and subchondral bone, other articular tissues, including the meniscus, should be targeted. Previous findings had shown that TGXTC was clinically effective in the treatment of KOA through multiple targets (14-20). However, whether TGXTC has a protective effect on meniscus remains unclear. In the present study, TGXTC was demonstrated to be able to efficiently reduce the destruction of the cartilage structure of the meniscus and improve the composition and function of the meniscus cartilage matrix.

In this experiment, the KOA model was induced by injecting 0.2 ml of 4% papain solution on days 1, 4 and 7. In addition, the Mankin score results showed that the model of early KOA was

successfully established. Quantitative analysis of the calcified area in meniscus showed that in the model group, the meniscus lost its normal shape, the surface layer of meniscal cartilage was damaged and the surface cells were absent, while the middle chondrocyte proliferation was obvious, which could be related to the cell stress proliferation response caused by the injury. In addition, the calcified area in meniscus was significantly increased, which could be related to the increase of calcified cells, leading to meniscal cartilage degeneration. In the TGXTC group, the structure of meniscus gradually returned to normal shape, and the abnormal proliferation of the middle chondrocyte and the area of calcified area was significantly reduced, which indicated that TGXTC could improve the structure of meniscus, then reduce the brittleness of meniscus and restore its elasticity.

Effect of TGXTC on the proteoglycan synthesis of meniscus. The central region of the meniscus is similar to articular cartilage, which is also hyaline cartilage containing collagen protein, proteoglycan and water molecules, but the composition ratio of the two components is not the same (36). Previous findings have shown that meniscus injury is associated with the process of KOA. When KOA occurs, meniscus degeneration will occur progressively, resulting in corresponding changes in the composition and morphology of meniscal hyaline cartilage (37).

In the TGXTC group, the content of proteoglycan in meniscal cartilage increased significantly, indicating that TGXTC could promote the synthesis and secretion of proteoglycan in meniscal chondrocyte, increase the content of cartilage matrix components and enhance the ability of binding water, then increase the elasticity of meniscus, and finally improve the function of the meniscus.

Effect of TGXTC on the ultrastructure of meniscus. In the present study, we found that in the early stage of KOA, there were fewer chondrocyte processes, fewer organelles, and organelles synthesizing proteins and energy-supplying became swelling and degeneration in the meniscus, which indicated that cells and organelles degenerated. The synthesis and secretion function of cells was weakened, which reflected that collagen fibrils around cells became thin, sparse and disorderly arranged.

Following treatment with TGXTC, cell processes increased, organelles in cytoplasm decreased, swelling and degeneration were restored, glycogen accumulated was consumed, and extracellular collagen fibril morphology was restored, which indicated that TGXTC could alleviate the degeneration of chondrocytes and organelles in early osteoarthritis meniscus of rats, restore the function of oxidative productive organelles, and improve the function of cell synthesizing and secreting proteins.

In conclusion, TGXTC may decrease meniscus fragility by reducing the area of calcified area and increase the water binding capacity and elasticity by promoting the synthesis and secretion of proteoglycan in the meniscus cartilage matrix. Therefore, TGXTC could improve meniscus function by reducing the degree of meniscal cartilage lesion and improving meniscal cartilage structure. Subsequently, TGXTC could be used in the treatment of meniscus injury in early osteoarthritis, so as to control the occurrence and development of osteoarthritis.

Acknowledgements

Not applicable.

Funding

This study was supported by the Natural Science Foundation of Fujian Province (grant no. 2015J01690).

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

WC and CT conceived and designed the study, and reviewed and edited the manuscript. X Lu and FF performed the drug intervention and model replication experiment. JC, X Lu and MH performed the histology experiment. GW and YH evaluated the degeneration of cartilage structure by modified Mankin scoring principles. X Lu, X Liu, RL and ZL performed the transmission electron microscopy experiment. YH, X Liu and WC performed the microscopic image analysis. GW and YH designed the experiment and wrote the manuscript. All authors read and approved the final version of the manuscript.

Ethics approval and consent to participate

All experimental rats and procedures were approved by Animal Care and Usage Committee of Fujian University of Traditional Chinese Medicine (Fuzhou, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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